RESEARCH ARTICLE

Variation in plant traits and phylogenetic structure associated with native and nonnative species in an industrialized flora

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Abstract

Industrialized sites are hotspots for nonnative species because of continuous anthropogenic disturbance and nonnative propagule rain resulting from hitchhikers exchanged through global trade. Investigating plant traits and the phylogenetic structure of species at initial ports of entry can contribute to our understanding of how species are introduced to, assembled into, and survive at industrialized sites, which can also inform how susceptible these sites are to nonnative plant invasions. To compare native and nonnative species, we asked three questions: 1) Are plant traits differentially associated with species nativity (native versus nonnative)? (2) Do these traits have phylogenetic signals? and (3) What is the phylogenetic structure of each trait for native and nonnative species? We collected, identified, and vouchered 170 angiosperm species within the Garden City Terminal at the Port of Savannah, Georgia, USA, the largest container terminal in North America. Species nativity was derived from the literature, as were traits of pollination syndrome, dispersal syndrome, duration, and growth habit. Pearson's Chi-squared tests were used to determine if traits were differentially associated with species nativity. Phylogenetic signal, along with mean pairwise distance (MPD) and mean nearest taxon distance (MNTD), were used to assess the degree of phylogenetic relatedness of native and nonnative species with each trait. Nonnative species showed a significant association with multiple pollination syndromes. Native species were significantly associated with perennial duration and zoophily pollination syndrome. All traits possessed a phylogenetic

signal, and the anemophily pollination syndrome was significantly clustered for both native and nonnative species. Still, most other traits differed in their phylogenetic structure pattern based on the nativity. Overall, findings suggest that the environment is filtering for native and nonnative species that possess traits promoting introduction and survival at this industrialized point-of-entry. They also suggest that nonnative species trait differences partition available niches that promote their introduction to the site. More research is needed at industrialized sites to inventory and monitor the floristic community, investigate the establishment and spread probabilities of nonnative species, and prevent and mitigate nonnative species risks and impacts.

Keywords
global trade, industrialized flora, phylogenetic structure, species nativity, trait associations

Introduction

Approximately 13% (47,840) of all vascular plant species have the potential to become nonnative species in new environments (Seebens et al. 2018). As the world becomes increasingly connected and the introduction of nonnative species accelerates, there is an increasing need to prevent and mitigate risks of potentially successful species invasions through early-detection and rapid-response (**EDRR**) efforts (Bezeng et al. 2013; Seebens et al. 2017; Pyšek et al. 2020; Seebens et al. 2021). Upon arrival at a novel range, nonnative species may establish after the transport and introduction phases of the biological invasion framework (Blackburn et al. 2011; Harris et al. 2018). These new arrivals, commonly pre-adapted to disturbance and ruderal in nature (Davis et al. 2000), can then spread in a new terrestrial landscape of varied niches formed by human activities (Cadotte and Lovett-Doust 2001; Dolan et al. 2017; Borden and Flory 2021). Investigating plant traits of currently present nonnative species and mapping those traits onto a phylogeny to compare with established native species are key to improving our understanding of how the environment and human-disturbance in new landscapes can select for certain traits. Results from studies comparing traits and phylogenies between native and nonnative species can then be used to inform assessments of industrialized flora (Lucardi et al. 2020a) and to predict nonnative species' capacity to become invasive at initial introduction sites (Lucardi et al. 2020b).

Industrialized sites experience extraordinarily high levels of human activity and disturbance, creating environmental constraints that limit plant occupancy (Williams et al. 2009; Kowarik and von der Lippe 2018; Knapp et al. 2022). Heavily and continuously disturbed industrialized sites (immature novel ecosystems, as defined by Kowarik and von der Lippe 2018) are generally open-canopy locales with intense solar radiation, pollutants, and limited substrate availability. Often, these human-dominated sites are linked to the introduction of nonnative species (Hobbs and Huenneke 1992; Crawley et al. 1996; Cadotte and Lovett-Doust 2001; Daehler 2003; Tsang et al. 2019; Lucardi et al. 2020b). However, both native and nonnative species arriving in industrialized environments may require specific traits conducive to their survival and

potentially longer-term establishment that for nonnative species may result in invasive spread (Funk and Vitousek 2007; Cadotte et al. 2017; Borden and Flory 2021).

In an industrialized flora (Lucardi et al. 2020a), traits deemed important for the successful introduction and survival of native and nonnative species include dispersal and pollination syndromes, growth habit, and duration (lifespan) (Pyšek et al. 1995; Pyšek et al. 2008; Borden and Flory 2021). Certain strategies involving these traits have been previously linked to the ability of plant species to occupy and survive in urban areas and industrialized sites (Williams et al. 2015; Palma et al. 2017; Knapp et al. 2022). For example, species with wind dispersal and wind pollination (Williams et al. 2015; but see Palma et al. 2017) have been found at higher frequencies in urban habitats than species with other dispersal mechanisms; this may be due to increased air turbulence (Knapp et al. 2010) and the suitability of these habitats to other dispersal and pollination mechanisms (Lososová et al. 2006). Frequently, disturbed sites are associated with annual species (Palma et al. 2017), whose lifespans are less affected by disturbances than biennial and perennial species (Knapp et al. 2022). Interestingly, in studies that have compared the traits of native and nonnative species, some have found similar or shared traits (Leishman et al. 2010; Tecco et al. 2010; Lemoine et al. 2015), whereas others have found dissimilar traits linked to species nativity (Pyšek and Richardson 2008; van Kleunen et al. 2010; Godoy et al. 2011).

Uncertainty surrounding which traits are linked to species' success in new environs limits understanding of how invasion happens and how to predict it (Ehrenfeld 2010; Drenovsky et al. 2012; Catford et al. 2019; Palma et al. 2021). Previous studies have highlighted wind pollination (Andersen 1995), fleshy fruits (Andersen 1995), and taller plant height (Crawley et al. 1996; Williamson and Fitter 1996; Montesinos 2021) as important plant traits for invasion success, but other studies have found contrasting results. For example, some research has noted a stronger link between nonnative species and animal or self-pollination than wind pollination (Williamson and Fitter 1996; Cadotte and Lovett-Doust 2001; Milanović et al. 2020). Investigation of key traits that promote the introduction and survival of nonnative species at initial arrival sites could illuminate the formation of species composition at industrialized or urban sites. Nonnative species traits should also be compared to the traits of native species to address the similarities or differences among those in high-disturbance, industrialized environments (Funk and Vitousek 2007; Loiola et al. 2018).

Analyses of phylogenetic signal and structure can be used to understand which traits facilitate the assembly of native and nonnative species in industrialized communities. For instance, the presence of phylogenetic signals would indicate the degree to which phylogenetic similarity predicts trait similarity in the community (Yang et al. 2014). Phylogenetic structure analyses can highlight the phylogenetic distribution of native and nonnative species with certain traits in the community (Loiola et al. 2018). For example, the finding of an over-dispersed pattern for a trait may highlight niche partitioning of species along the trait axis (Funk et al. 2008; Liu et al. 2013; Lemoine et al. 2015). A clustered pattern for a trait may indicate habitat filtering that favors certain traits over others (Funk et al. 2008; Liu et al. 2013; Lemoine et al. 2015) or

performance differences between species (Mayfield and Levine 2010). These phylogenetic patterns can highlight if traits of native and nonnative species have similar or dissimilar phylogenetic distributions, plant traits or lineages favored at industrialized sites, and the potential processes that lead to their assembly at these sites (Dolan et al. 2017).

Industrialized sites, such as the Garden City Terminal, the primary container handling facility of the Port of Savannah, present an ideal laboratory to investigate traits and phylogenetic distributions of native and nonnative species under continuous, active disturbance. At the Garden City Terminal's green spaces, Lucardi et al. (2020a) presented the stark contrast between plant species richness of native and nonnative species. They found that nonnative species richness and proportional nonnative to native ratios at this industrialized site were higher when compared to other floras in the same region. Herein, we addressed the following questions from the vascular plants collected in Lucardi et al. (2020a): (1) Are traits differentially associated with species nativity? (2) Do these traits have phylogenetic signals? and (3) What is the phylogenetic structure of each trait for native and nonnative species? We predict that traits vary in their association with native and nonnative species, traits have phylogenetic signals, and patterns of phylogenetic structure of species' traits differ between native and nonnative species. Based on these questions, we hope to determine how trait differences contribute to the introduction of nonnative species at this industrialized site.

Methods

Study site

This study was conducted at the Port of Savannah, Georgia, USA (32°07.3'N, 81°08.4'W). At the port, we specifically focused on the Garden City Terminal (**GCT**), the main container-handling terminal that spans 485.6 hectares and is primarily composed of impervious surfaces (i.e., asphalt) interspersed with small green spaces for water run-off (Lucardi et al. 2020a). The Port of Savannah has a subtropical climate and is in the USDA plant hardiness zone 8b (USDA Plant Hardiness Zone Map 2012). This hardiness zone is characterized by average annual minimum temperatures ranging from -9.4 °C to -6.7 °C (Daly et al. 2012). During the sampling period (2015–2017), the average temperature was 21.1 °C (NOAA 2021).

Floristic and trait inventory

The flora was sampled from six green spaces (4.51 ha, ~1% of the GCT) on four separate occasions between August 2015 and February 2017 to capture seasonal changes in the flora (see Lucardi et al. 2020a for details). All accessible angiosperm species in flower or fruit were collected during each sampling date. Vouchers from these repeated surveys were identified, and dried specimens were deposited into the Arkansas State University Herbarium (**STAR**) and Columbus State University Herbarium (**COLS**). Our collection comprises 174 species, representing 130 genera and 51 families. To

make all investigated plant traits and phylogenetic structure patterns comparable, we only consider angiosperms (170 species), removing two fern species, *Asplenium platyneuron* (L.) Britton, Sterns & Poggenb and *Lygodium japonicum* (Thunb.) Sw., a notable nonnative and pyrogenic invasive plant, along with two gymnosperm species (*Juniperus virginiana* L. and *Pinus taeda* L.).

Information on traits was gathered from the literature (Fig. 1; Suppl. material 1). Nativity (Native/Nonnative) was previously reported in Lucardi et al. (2020a) for this flora. The other four traits included 1) dispersal syndrome, 2) pollination syndrome, 3) duration, and 4) growth habit. Most of this information was gathered from the USDA PLANTS Database (USDA NRCS 2021). Missing information was supplemented by the literature for any particular species (Suppl. material 1). All traits are categorical. Dispersal syndrome was composed of five categories, according to Schulze et al. (2019): anemochory (wind dispersal), autochory (self-dispersal), hydrochory (water dispersal), zoochory (animal dispersal), and polychory (multiple dispersal mechanisms). Pollination syndrome was also composed of five categories, according to Yang et al. (2020): anemophily (wind pollination), hydrophily (water pollination), self-pollination, zoophily (animal pollination), and multiple (multiple pollination mechanisms). There were four duration categories: annual, biennial, perennial, and multiple (multiple duration strategies). Lastly, there were six categories of growth habit: forb, graminoid, subshrub, tree, vine, and multiple (multiple growth strategies). Duration and growth habit categories were defined by those on the USDA PLANTS Database (USDA NRCS 2021).
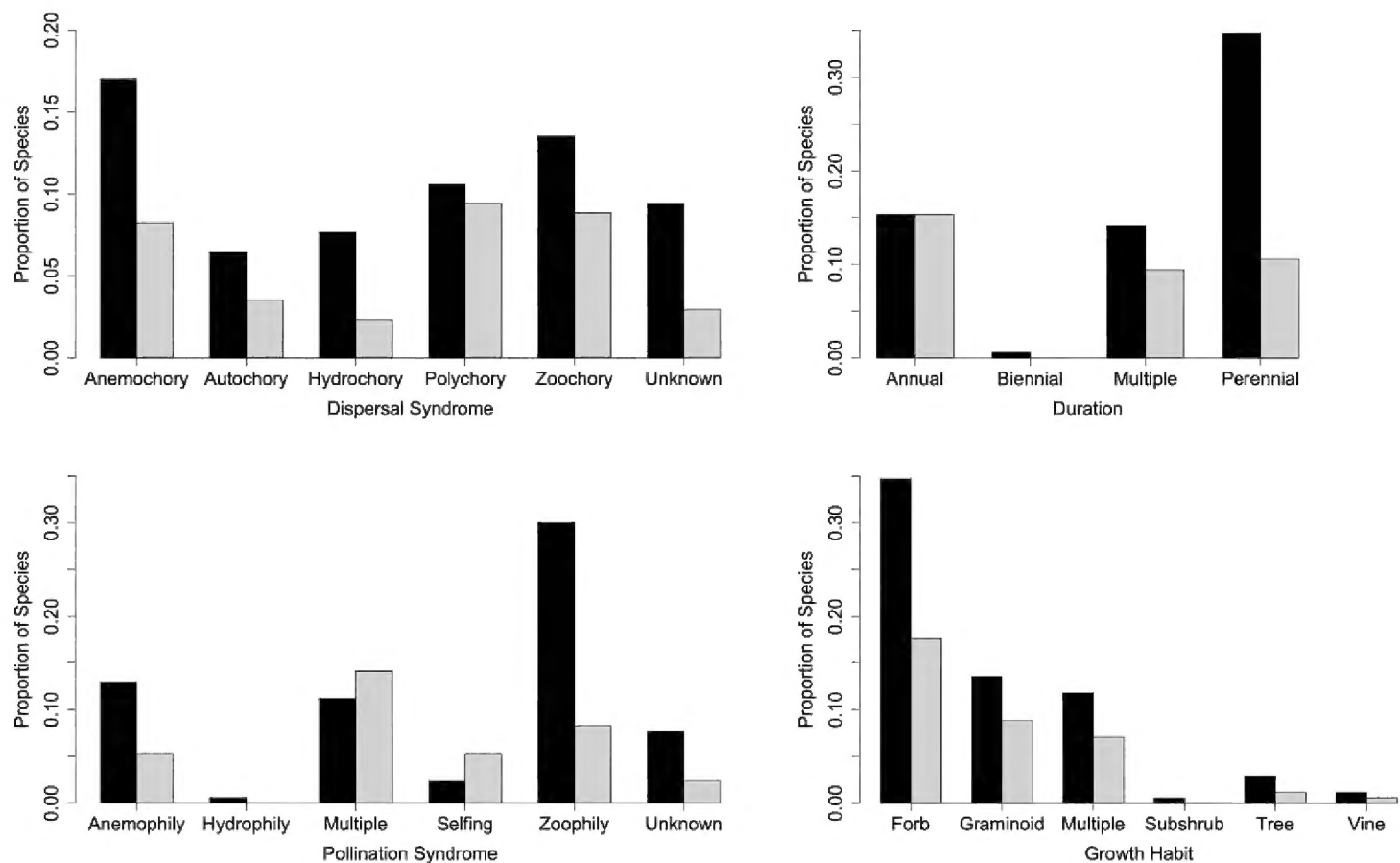

Figure 1. The proportion of all species in the dataset in each category of each trait investigated. The flora consists of 170 species comprised of 110 native and 60 nonnative species. Black bars represent the proportion of native species and gray bars represent the proportion of nonnative species in all figure panels.

Trait data analysis

Pearson's Chi-squared tests were used to determine if traits were differentially associated with species nativity, with the null hypothesis assuming independence of traits and nativity. For analyses exhibiting significant differences, we performed *post hoc* analyses considering all combinations of trait categories and species nativity using the chisq.posthoc.test package (Ebbert 2019) in R statistical software (R Core Team 2021). Categories with low sample sizes were eliminated from analyses, including removing biennial (1) from the duration trait, hydrophily (1) from the pollination syndrome trait, and subshrub (1), vine (3), and tree (7) categories from the growth habit trait.

DNA barcode analysis: DNA isolation, PCR amplification, and sequencing

DNA barcoding of the flora was performed to build a phylogenetic tree for analyses and for confirmation of species identifications (Whitehurst et al. 2020). Leaf samples were taken from each plant specimen and stored on silica gel until processing. All DNA barcode analyses were conducted at the Canadian Centre for DNA Barcoding (**CCDB**), Biodiversity Institute of Ontario, Canada, including DNA extraction (Ivanova et al. 2008; Ivanova et al. 2016), PCR amplification (Kuzmina and Ivanova 2011; Fazekas et al. 2012), and sequencing (Ivanova and Grainger 2006; Kuzmina and Ivanova 2006). Ribulose-bisphosphate/carboxylase large subunit (*rbc*L) and maturase-K (*mat*K) gene regions of the chloroplast genome were sequenced using forward and reverse primers and Sanger sequencing technology (Levin et al. 2002; Kress et al. 2009). In instances where sequencing failed, publicly available sequences were used, when available, from the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007) or GenBank (Benson et al. 2013). All sequences generated in this study are publicly available on BOLD and GenBank (Suppl. material 2). Only species with both DNA barcode gene regions were included in phylogenetic analyses. We eliminated nine species that either (1) amplified only the *rbc*L or *mat*K region or (2) failed to sequence completely (Suppl. material 2), resulting in 161 plant species for inclusion in phylogenetic analyses. Sequences for an additional two species placed them into questionable areas of the phylogenetic tree, causing us to question their tissue sample used in sequencing, so we eliminated them from phylogenetic analyses. The final dataset for phylogenetic analyses consisted of 159 species (Suppl. material 3).

Phylogenetic tree reconstruction

Sequences of the *rbc*L and *mat*K gene regions were aligned separately using multiple alignment and fast Fourier transform (MAFFT v 7.471) with the FFT-NS-2 algorithm (Katoh and Standley 2013). The alignments were then concatenated into a super-

matrix from which a phylogeny was generated. A phylogeny was constructed using maximum likelihood (ML) methods in the "phangorn" package (Schliep 2011) in R statistical software (R Core Team 2021) with *Ginkgo biloba* L. as the outgroup. Nucleotide substitution was modeled using the general time-reversible substitution model with gamma-distributed rate variation and a proportion of invariant sites (GTR + Γ + I). Node support was estimated from 1000 bootstrap replications.

Phylogenetic signals and structure

We quantified phylogenetic signals in the four traits to determine the degree to which the phylogenetic tree estimates the similarity of species traits. Phylogenetic signals were determined by quantifying the parsimony Sankoff score calculated from the distribution of trait categories on the phylogeny (Maddison and Slatkin 1991) using the "phangorn" package (Schliep 2011) in R statistical software (R Core Team 2021). The significance of the Sankoff score was determined by randomly shuffling the species on the tips of the phylogeny 999 times to generate a null distribution that was compared to the observed parsimony score to calculate a P-value. A P-value < 0.05 was indicative of closely related species having similar traits.

   We also calculated the mean pairwise distance (**MPD**) and mean nearest taxon distance (**MNTD**) using the "picante" package (Kembel et al. 2010) in R statistical software (R Core Team 2021). These metrics measure the degree of phylogenetic relatedness of species in defined groups. In this study, groups were made comparing native and nonnative species groups for each trait category. For example, for the dispersal syndrome trait, species were grouped into native or nonnative for each of the categories (anemochory, autochory, hydrochory, zoochory, and polychory). In this instance, MPD is the mean phylogenetic distance among all pairs of species within a group and captures the overall phylogenetic dissimilarity of the taxa in the group of interest (Swenson 2014). The mean distance between each species within a group and its closest relative is expressed as MNTD. The two metrics provide complementary information for which MPD is focused on the whole group phylogeny, and MNTD captures finer scale phylogenetic patterns, making it more sensitive to sister-taxa distances and the length of the tips of the phylogeny (Swenson 2014; Cadotte et al. 2018; Loiola et al. 2018).

   For MPD and MNTD, observed values were compared to null distributions generated by randomizing the names of the taxa on the phylogenetic distance matrices 999 times to calculate standardized effect sizes (SES) and P-values (quantiles). Negative SES values (obs.z < 0) and low quantiles (obs.p < 0.05) for both MPD and MNTD indicated species in a group are phylogenetically clustered, with smaller phylogenetic distances among the species in the group than expected (Swenson, 2014). Positive SES values (obs.z > 0) and high quantiles (obs.p > 0.95) indicated species in a group are phylogenetically over-dispersed, with greater phylogenetic distances among species in the group than expected (Swenson 2014).

Results

Relationships between traits and species nativity

Of the 170 species in this study flora, 110 were native to the southeastern region of the USA, and 60 were nonnative (Appendices S1 and S3). Significant differences in association were found between two of the four traits and species nativity (Fig. 1, Table 1). Pollination syndrome resulted in a significant association with species nativity (χ^2 = 19.867, df = 3, P < 0.01). *Post hoc* analysis revealed significant associations between the categories multiple (P = 0.02) and zoophily (P = 0.01), and species nativity (Table 1). The multiple pollination strategy was strongly positively associated with nonnative species, and zoophily was strongly positively associated with native species. Duration was also significantly associated with species nativity (χ^2 = 10.07, df = 2, P < 0.01). The perennial (P = 0.02) duration category was significantly associated with species nativity according to *post hoc* analysis (Table 1). Nonnative species had a strong positive association with annual duration and a strong negative association with perennial duration. Native species showed opposite and weaker association patterns with annual and perennial duration categories compared to nonnative species. The overall χ^2 score was largely influenced by annual (31%) and perennial (32%) nonnative species. These categories contributed most to the overall χ^2 score because they had far fewer observed species than expected.

Dispersal syndrome was not significantly associated with species nativity (χ^2 = 3.29, df = 4, P = 0.51). However, there were still differences in the signs of association between trait categories and species nativity. Native species had positive associations with anemochory and hydrochory dispersal syndromes but negative associations with

Table 1. Representation of association found between traits and species nativity (native and nonnative). Significant associations (bolded) were determined from Pearson's Chi-squared tests with *post hoc* analyses. The sign of association between each trait category and nativity are given, negative (–) and positive (+). Trait categories with significant phylogenetic structure are also displayed. Trait categories with significant phylogenetic clustering are denoted by ^. Trait categories with significant phylogenetic over-dispersion are denoted by *.

Trait	Trait Category	Native	Nonnative
Dispersal Syndrome	Anemochory	+ ^	–
	Autochory	+	– ^
	Polychory	– *	+ *
Pollination Syndrome	Anemophily	+ ^	– ^
	Multiple	–	+
	Selfing	–	+ *
	Zoophily	+ ^	–
Duration	Multiple	– ^	+
	Perennial	+ ^	–
Growth Habit	Forb	+ ^	– ^
	Graminoid	– ^	+ ^
	Multiple	– ^	+

polychory and zoochory dispersal syndromes. Nonnative species had opposite associations with the dispersal syndrome categories as native species. Overall, polychory (native: 17%; nonnative: 29%) contributed the most to the total x^2 score. There was also no significant association between growth habit and species nativity (x^2 = 0.59, df = 3, P = 0.90). The graminoid category contributed the most (native = 18%, nonnative = 32%) to the overall x^2 score.

Phylogenetic signals and structure

We quantified the observed parsimony Sankoff score for each trait and compared it to a null distribution of parsimony scores to determine significance. We found significant phylogenetic signals for all four traits (pollination and dispersal syndromes, growth habit, and duration), with close relatives generally sharing more similar traits than expected by chance (Table 2). Phylogenetic structure analyses investigated the structure of native and nonnative species within each category of each trait (Table 3). For native species, anemochory and polychory dispersal syndromes showed significant phylogenetic structure. The standardized effect sizes of MPD and MNTD showed evidence of phylogenetic clustering for anemochory, whereas MNTD showed evidence of over-dispersion for polychory (Tables 1, 3). Standardized effect sizes of MNTD also showed evidence of over-dispersion for polychory for nonnative species (Tables 1, 3). Autochory dispersal syndrome was found to be significantly, phylogenetically clustered for nonnative species according to MPD and MNTD (Tables 1, 3). Standardized effect sizes of MPD and MNTD supported significant phylogenetic clustering for zoophily, while MNTD supported significant phylogenetic clustering for the anemophily categories of pollination syndrome in native species (Tables 1, 3). For nonnative species, standardized effect sizes of MPD and MNTD showed significant phylogenetic clustering for anemophily, where MNTD supported significant phylogenetic over-dispersion for the selfing category of pollination syndrome (Tables 1, 3). Only native species showed any significant phylogenetic structure for the duration (lifespan) trait, with MPD and MNTD showing evidence of phylogenetic clustering for multiple and perennial duration categories, respectively (Tables 1, 3). Finally, for the growth habit trait for native species, significant phylogenetic clustering was supported by MPD and MNTD for the forb and graminoid categories, while MPD provided support for the multiple growth habit category (Tables 1, 3). Significant phylogenetic clustering was also supported for the forb (MPD) and graminoid (MPD and MNTD) categories based on standardized effect sizes for nonnative species (Tables 1, 3).

Table 2. Phylogenetic signals using parsimony Sankoff scores. All P-values were significant (< 0.05).

Traits	Sankoff	n	P
Dispersal Syndrome	84	139	<0.01
Pollination Syndrome	69	142	<0.01
Duration	81	159	<0.01
Growth Habit	60	158	<0.01

Table 3. Phylogenetic structure within each of the categories of each of the four traits. MPD.obs.z is the standardized effect size of the mean pairwise distance measurement. MNTD.obs.z is the standardized effect size of the mean nearest taxon distance measurement. Standardized effect sizes were calculated from comparisons of observed values to null distributions generated by randomizing the names of the taxa in the phylogenetic distance matrices 999 times. P-values in bold are significant. Significant phylogenetic clustering is denoted by ^ ($P < 0.05$). Significant phylogenetic over-dispersion is denoted by * ($P > 0.95$).

Trait	ntaxa	MPD.obs.z	P	MNTD.obs.z	P
Dispersal Syndrome					
Anemochory	24	-7.19	**0.001^**	-2.40	**0.012^**
Anemochory.NN	14	0.27	0.572	-0.92	0.178
Autochory	11	-0.73	0.226	0.46	0.679
Autochory.NN	6	-1.95	**0.049^**	-1.88	**0.034^**
Hydrochory	12	0.98	0.855	-0.84	0.199
Hydrochory.NN	4	0.52	0.660	-0.79	0.767
Polychory	18	0.93	0.812	1.99	**0.972***
Polychory.NN	15	2.23	**0.997***	-0.38	0.365
Zoochory	22	-1.47	0.080	-1.24	0.110
Zoochory.NN	13	0.80	0.788	-0.99	0.169
Pollination Syndrome					
Anemophily	18	0.51	0.683	-2.72	**0.004^**
Anemophily.NN	9	-4.25	**0.001^**	-2.19	**0.016^**
Multiple	18	-0.41	0.322	0.66	0.746
Multiple.NN	24	0.22	0.542	-1.14	0.130
Selfing	4	0.70	0.721	1.23	0.883
Selfing.NN	9	2.04	**0.994***	0.49	0.676
Zoophily	47	-5.47	**0.001^**	-1.98	**0.024^**
Zoophily.NN	13	-1.71	0.057	0.43	0.657
Duration					
Annual	27	-1.23	0.124	-1.57	0.060
Annual.NN	25	1.18	0.885	-1.27	0.115
Multiple	22	-3.72	**0.002^**	0.20	0.576
Multiple.NN	16	1.30	0.927	-0.41	0.334
Perennial	52	-0.23	0.383	-1.95	**0.025^**
Perennial.NN	17	-0.201	0.377	0.16	0.556
Growth Habit					
Forb	56	-7.34	**0.001^**	-2.34	**0.009^**
Forb.NN	29	-2.00	**0.035^**	-1.15	0.131
Graminoid	18	-4.69	**0.001^**	-4.75	**0.001^**
Graminoid.NN	15	-6.87	**0.001^**	-3.85	**0.001^**
Multiple	20	-2.95	**0.007^**	-1.23	0.112
Multiple.NN	10	-0.64	0.253	-0.12	0.446
Tree	5	-1.77	0.052	-0.37	0.360
Tree.NN	2	-0.57	0.268	-0.64	0.246
Vine	2	-1.27	0.101	-1.23	0.106
Vine.NN	1	NA	NA	NA	NA

Discussion

This research is part of an ongoing, innovative research initiative to quantify and assess plant communities within industrialized initial points-of-entry sites (Lucardi et al. 2020a), and to directly measure the diversity and phenology of propagule pressure and model risk of establishment (Lucardi et al. 2020b). Here, we investigated 1) the traits

of native and nonnative plant species from the industrialized flora located at the GCT of the Port of Savannah, Georgia, USA, 2) quantified associations between species traits and their nativity (native or nonnative), and 3) evaluated phylogenetic structure patterns of these traits. Consistent with our first prediction, we found that native and nonnative species were associated with different traits. Importantly, nativity was significantly associated with different categories of pollination syndrome and duration. We also found that all traits had phylogenetic signals, supporting our second prediction. Lastly, we found mixed support for our third prediction, as all but two trait categories with significant phylogenetic structure differed between native and nonnative species.

Native species in the flora at the Garden City Terminal of the Port of Savannah showed significant, positive associations with perennial duration and zoophily pollination syndrome (Table 1). Previous research into these species at this site also noted their adaptation to weedy and disturbed habitats (based on Coefficients of Conservatism [CofC], Lucardi et al. 2020a). Research into the southwestern Ontario flora, with 27% nonnative species, previously found anemochory, zoophily, and perennial duration to be associated with native species (Cadotte and Lovett-Doust 2001). Conversely, Flores-Moreno et al. (2013) found anemochory and hydrochory dispersal syndromes to be strongly associated with nonnative rather than native species in a global assessment. Discrepancies between our study and their global assessment could be due to the nature of the industrialized flora studied here, again stressing the importance of differentiating between comparing the traits of native and nonnative species in natural versus industrialized or human-dominated sites and among different habitats and environmental conditions more broadly (Milanović et al. 2020).

Nonnative species only showed a significant, positive association to multiple pollination syndromes (Table 1). In general, pollination syndrome is an important trait determining the invasive potential of plants (Gassó et al. 2009), with previous studies finding a variety of pollination syndromes associated with nonnative species, including wind (Andersen 1995), animal (Williamson and Fitter 1996), and self-pollination (Milanović et al. 2020). Similarly, our findings suggests that instead of having one particular pollination syndrome, having multiple pollination syndromes may better benefit the introduction of nonnative species at our study site. Recently, we documented previously undetected hitchhiking species on the air-intake grilles of refrigerated shipping containers and primarily found wind-dispersed, nonnative graminoids entering the Port of Savannah via maritime trade (Lucardi et al. 2020b). Though not all of these inadvertently moved propagules will result in plant establishments, the intensity of the propagule pressure at this industrialized site further underscores the immediate need to target such areas with stronger prevention and interception programs and extensive EDRR (Burk 1877; Lucardi et al. 2020b).

As highlighted above, we found differences in the traits associated with native versus nonnative species, notably, significant differences in associations of nativity with pollination syndrome and duration (Table 1). This trend contrasts with previous research that found no difference in species' traits with different nativity (Thompson et al. 1995; Leishman et al. 2010; Tecco et al. 2010). Pyšek et al. (1995) did not find a significant difference between pollination syndromes of native and nonnative species in

a Czech flora. Still, they did see a significant difference in dispersal agents. Importantly, the findings of Pyšek et al. (1995) were only apparent when native and nonnative species were examined in anthropogenic habitats compared to natural environments. The findings of Pyšek et al. (1995) and Wolf et al. (2020) highlight the context-dependency associated with the influence of human-dominated systems, such as industrialized and urban floras, and the uniqueness of the species in these floras as compared to natural sites. For instance, the flora analyzed in this study is composed of approximately 35% nonnative species, a higher percentage than found in 19 other comparison floras published since 1990 in Georgia and South Carolina, USA (Lucardi et al. 2020a). Interestingly, a recent study introducing the Biotic Novelty Index (Schittko et al. 2020) showed that biotic novelty increased due to increasing nonnative species and increasing impervious surfaces, both of which reflect the situation at industrialized sites like the green spaces of the GCT of the Port of Savannah.

We also found phylogenetic signals for all four traits assessed in this study. In other words, closely related species shared more similar traits more often than expected by chance in the industrialized flora at the Garden City Terminal at the Port of Savannah (Table 2). These findings are not surprising given the wide distribution of nonnative species in the phylogeny of this flora, each with many close native relatives (Suppl. material 3). However, we found that native and nonnative species had different traits with different phylogenetic structure patterns at this site (Tables 1, 3). Anemophily pollination syndrome and polychory dispersal syndrome were the only trait categories where both native and nonnative species had significant phylogenetic structure. Both native and nonnative species showed significant phylogenetic clustering for anemophily, suggesting that the environment of this industrialized site favors species with anemophily over other pollination syndromes. Polychory was significantly phylogenetically over-dispersed for native and nonnative species, suggesting species may specialize in different dispersal syndromes that allow them to partition niche space at this site.

Overall, the categories of traits showing a significant phylogenetic structure in the flora, for the most part, differed from those that had significant relationships with species nativity (Table 1). Only traits of native species (zoophily pollination syndrome and perennial duration) showed significant associations with species nativity (native) and also had non-random patterns of phylogenetic structure (clustering). These results suggest a strong tendency of native species to have perennial duration and zoophily pollination syndrome, which may reflect the regional species pool and deserves future investigation.

Determining what makes communities invasion-prone has been elusive. There are intuitive arguments for environmental filtering, whereby nonnative plants should have traits similar to native ones, and empty niche or niche partitioning, whereby nonnative plants should have different traits from native ones (Elton 1958; Bezeng et al. 2013; Hulme and Bernard-Verdier 2018; Enders et al. 2020). Our findings suggest that both mechanisms are in play at this industrialized study site. Phylogenetic structure patterns and differences in the associations of traits among native

and nonnative species suggest that niche partitioning facilitates the introduction and survival of nonnative species at our site (MacDougall and Turkington 2005). Complementing these differences are traits of nonnative species commonly associated with species in highly disturbed industrialized or urban floras, including annual duration, variation in pollination and dispersal syndromes (Williams et al. 2015; Palma et al. 2017). The environment of the industrialized flora at the Port of Savannah may be filtering for species with these traits. Based on findings in previous research, the introduction of native species at this site may have also been influenced by the environment as successfully occurring species show adaptation to weedy and disturbed habitats (Lucardi et al. 2020a). Similar findings to this study have been categorized as species needing similar traits when introduced to a novel community (environmental filtering) but also requiring distinct traits to disrupt the community (niche partitioning), as invaders are notoriously known for doing (Ordonez 2014; Divíšek et al. 2018). In the future, environmental filters associated with this human-dominated, highly disturbed site may further limit the traits and phylogenetic distribution of species within this site which may create a habit even more conducive to the introduction and survival of nonnative species (Williams et al. 2009; Bennett et al. 2014).

Despite the small amount of green space at the Garden City Terminal (~1% of the land area at the industrialized site), this industrialized flora is unique with a large number and proportion of nonnative species (Lucardi et al. 2020a). Many studies assessing species' traits in other floras have focused on other traits not available for most of the species in this study, limiting our ability to make a comparison with their findings. We also recognize that lower phylogenetic resolution may contribute to uncertainty in the phylogenetic analyses (Swenson 2009). However, 61% of branches in the phylogeny were highly supported (bootstrap support > 85%) and 68% were moderately supported (bootstrap support > 70%). In the future, additional DNA barcode regions would better help resolve the phylogeny. In the phylogenetic analyses, significant phylogenetic patterns were found for either MPD or MNTD, but not both metrics in some cases. These discrepancies may be due to the nature of these metrics, where MPD captures the overall phylogenetic dissimilarity of species in the group, whereas MNTD can detect finer scale phylogenetic patterns at the tips of the phylogeny that may be present (Erickson et al. 2014; Swenson 2014).

Conclusions

This research highlights differences in duration along with pollination and dispersal syndromes associated with species nativity that deserve consideration and further investigation in future studies of industrialized floras. It also highlights, through phylogenetic analyses, how highly disturbed sites may filter for species with traits such as anemophily pollination syndrome, regardless of species nativity. Finally, this research suggests the influence of environmental filtering and niche partitioning on the

similarity and dissimilarity, respectively, of nonnative and native species traits that may have allowed their introduction and survival at this site. More research, cooperation, and coordination are needed at industrialized and urbanized sites to more adequately investigate nonnative species' establishment and spread probabilities (Tsang et al. 2019; Lucardi et al. 2020b; Borden and Flory 2021). Regular monitoring at these sites is also essential for documenting spatiotemporal changes in plant and trait communities that favor the establishment of nonnative species through localized population dynamics and the hosting of newly arriving propagules.

Data availability

The GenBank accession numbers for all successfully sequenced *rbc*L and *mat*K DNA barcodes can be found in Suppl. material 2. Sequences are also publicly available on the Barcode of Life Data System (**BOLD**). Alignments used to generate the phylogenetic tree and the phylogeny in Suppl. material 3 along with all code can be found at https://doi.org/10.5281/zenodo.7101888.

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Author contributions

SJW, TDM, RDL, and KSB were involved in the conception of the idea. SJW, LEW, RDL, and KSB collected data. TDM identified the species morphologically. LEW prepared specimens for DNA barcode analysis, SJW analyzed data, and SJW wrote the original draft with edits, comments, and approvals from all authors.

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Supplementary material 1

Species included in this study, listed alphabetically, along with their traits
Authors: Samantha J. Worthy, Travis D. Marsico, Rima D. Lucardi, Lauren E. Whitehurst, Kevin S. Burgess
Data type: docx file
Explanation note: Species included in this study, listed alphabetically, along with their traits. All species are represented by vouchers stored at Arkansas State University Herbarium (STAR) and Columbus State University Herbarium (COLS). If citations are not included, the information was gathered from the USDA PLANTS Database (USDA, NRCS, 2021).

Link: https://doi.org/10.3897/neobiota.77.87307.suppl1

Supplementary material 2

Sequencing and collection information for the species in this study

Authors: Samantha J. Worthy, Travis D. Marsico, Rima D. Lucardi, Lauren E. Whitehurst, Kevin S. Burgess

Data type: docx file

Explanation note: Sequencing and collection information for the species in this study, listed alphabetically. The collection number and GenBank accession number for each sequence are presented below. Sequences downloaded from Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007) and GenBank (Benson et al. 2013) databases are noted with a * by the accession number. Only species with both DNA barcode gene regions were included in phylogenetic analyses, eliminating nine species that had either only a rbcL or matK sequence, noted by "Excluded" in the GenBank Accession column. Sequences for two species placed them into incorrect areas of the phylogenetic tree causing us to question their identity and eliminate them from phylogenetic analyses, noted as "Erroneous Sample" in the GenBank Accession column. Failed sequencing is noted in the sequence column and no GenBank accession number is given. All species are represented by vouchers stored at Arkansas State University Herbarium (STAR) and Columbus State University Herbarium (COLS).

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Supplementary material 3

Phylogenetic tree depicting genetic relationships among 159 species of the flora at the Port of Savannah, Savannah, Georgia, USA, with available sequences

Authors: Samantha J. Worthy, Travis D. Marsico, Rima D. Lucardi, Lauren E. Whitehurst, Kevin S. Burgess

Data type: docx file

Explanation note: Phylogenetic tree depicting genetic relationships among 159 species of the flora at the Port of Savannah, Savannah, Georgia, USA, with available sequences. The phylogeny was generated using maximum likelihood methods in the "phangorn" package (Schliep 2011) in R programming language (R Core Team 2021) using the general time-reversible model of substitution with gamma-distributed rate variation and a proportion of invariant sites (GTR + Γ + I) with Ginkgo biloba as the outgroup. Bootstrap values from 1000 replications are presented at each node. Tree topology was visualized using iTOL v. 6.3 (Letunic and Brok 2021). Species in black font are native, and species in blue font are nonnative.

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